

FIGURE 1

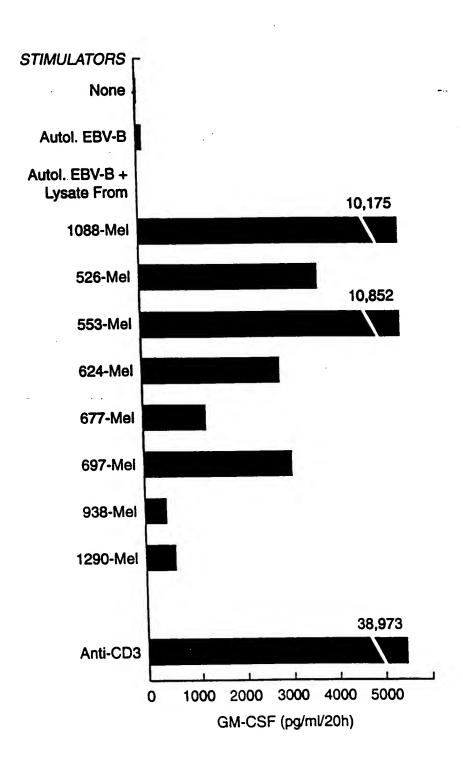


FIGURE 2

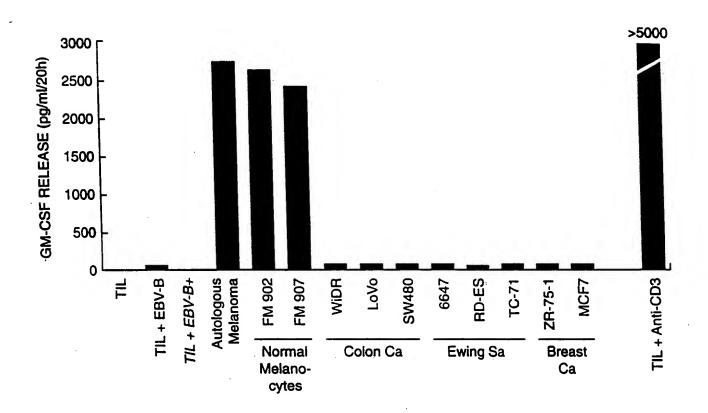


FIGURE 3

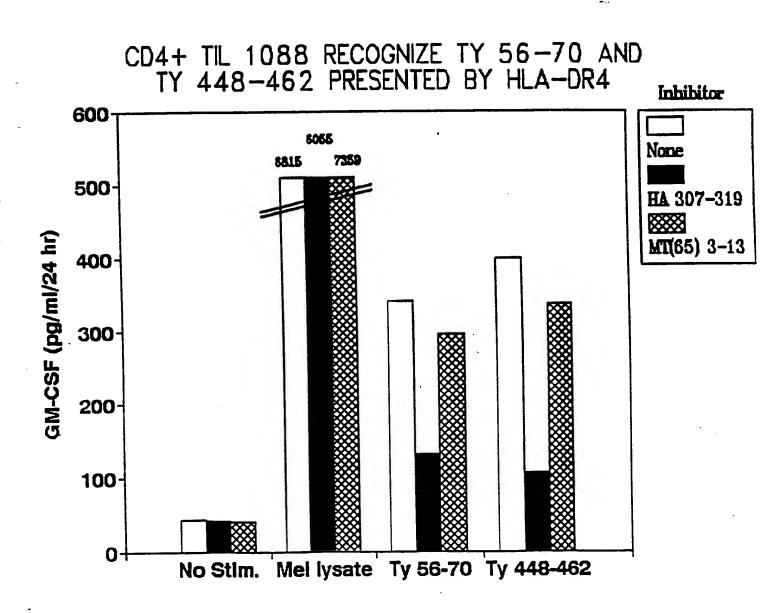


FIGURE 4

#### HLA Restriction of Tyrosinase Recognition by CD4<sup>+</sup> TIL 1088

(GM-CSF secretion, pg/ml/24 hr)

| Antigen<br>Presenting<br>Cells |      | Antigen Presented  |                     |                        |      |              |  |  |  |  |  |  |  |  |  |
|--------------------------------|------|--------------------|---------------------|------------------------|------|--------------|--|--|--|--|--|--|--|--|--|
|                                | None | 1088-mel<br>lysate | Ty 56-70<br>(A63→V) | Ty 448-462<br>(D456-V) |      |              |  |  |  |  |  |  |  |  |  |
|                                |      |                    | •                   |                        |      |              |  |  |  |  |  |  |  |  |  |
| Experiment 1                   |      |                    |                     |                        |      |              |  |  |  |  |  |  |  |  |  |
| 1088-EBV                       | 145  | 1179               | 2445                | 2969                   | 0301 | 0401         |  |  |  |  |  |  |  |  |  |
| 697-EBV                        | 282  | 1014               | >500                | 3815                   | 0401 | 1501         |  |  |  |  |  |  |  |  |  |
| BM14                           | 751  | 3282               | 4793                | 5121                   | 0401 | -            |  |  |  |  |  |  |  |  |  |
| 860-EBV                        | 376  | 870                | 383                 | 345                    | 0301 | 1301         |  |  |  |  |  |  |  |  |  |
| 583-EBV                        | 484  | 550                | 574                 | 477                    | 0404 | 1501         |  |  |  |  |  |  |  |  |  |
| 1087-EBV                       | 507  | 557                | 550                 | 566                    | 0701 | 1201         |  |  |  |  |  |  |  |  |  |
| Experiment 2                   |      |                    |                     |                        |      |              |  |  |  |  |  |  |  |  |  |
| 1359-EBV                       | 183  | 2238               | 1179                | 1714                   | 0301 | 0401         |  |  |  |  |  |  |  |  |  |
| BM14                           | 372  | 2822               | 1892                | 2805                   | 0401 | -            |  |  |  |  |  |  |  |  |  |
| 1485-EBV                       | 171  | 160                | 154                 | 222                    | 0407 | 15 <b>01</b> |  |  |  |  |  |  |  |  |  |
| COX                            | 544  | 482                | 735                 | 703                    | 0301 |              |  |  |  |  |  |  |  |  |  |

150

54

PGF

Antigen presenting cells pulsed with tumor lysate or 100 uM peptide overnight, then washed prior to assay, all cells at  $1\times10^6/ml$ 

76

46

#### FIGURE 5

1501

#### Determining the P1 and P6 Anchor Positions for Ty 56-70

|  |          |   |             |            |             |             |              |             |        |        |            |        |   |        |            |      | GM-CSF (pg/ml/24h.)        |                      |                 |  |  |
|--|----------|---|-------------|------------|-------------|-------------|--------------|-------------|--------|--------|------------|--------|---|--------|------------|------|----------------------------|----------------------|-----------------|--|--|
|  |          |   |             |            |             |             |              |             |        |        |            |        |   |        |            | Exp. | <u>1</u> -                 | Exp. 2               | Exp. 3          |  |  |
| Ty 56-70   | Q        | N | ı           | L          | L           | s           | N            | Α           | P      | L.     | G          | P      | Q | F      | P          | 29   | 95                         | 524                  | 903             |  |  |
| 158  | -        | - | Q<br>F<br>V | -          | -           | -           | -<br>-<br>-, | -           | -<br>- | -<br>- | -<br>-     | -      | - | -<br>- | -          | ;    | <8<br>39<br>ID             | <8<br>46<br>ND       | <8<br>ND<br>154 |  |  |
| L59  | •        | - | <u>.</u>    |            | -           | -           | -            | -<br>-<br>- | -      | •      | •          | •      | - | •      | -          | •    | <8<br><8<br>VD             | <8<br><8<br>ND       | <8<br>ND<br><8  |  |  |
| L60  | •        | - | -           | -          | Q<br>F<br>V | -           | -            | -           | -      | •      | -          | •      | - | -      | -          |      | <8<br>86<br>ND             | 62<br><8<br>ND       | <8<br>ND<br>10  |  |  |
| A63  | -        | - | -<br>-      | -<br>-     | -           | -           | -<br>-       | Q           | -      | -      | -<br>-     | -      | - | -      | -<br>-     | 8    | <8<br>8 <b>9</b>           | <8<br>2080           | ND<br>ND        |  |  |
| P64  | -        | - | -           | -          | -           | •           | -            | -           | Q<br>V | · -    | -          | -<br>- | - | -      | -          |      | <8<br><8                   | <8<br>48             | ND<br>ND        |  |  |
| L65  | -        | • | -<br>-      | . <b>.</b> | • •<br>• •  | -           | -            | •           | -      | C<br>V | ) -<br>/ - | -      | - | -      | . <u>-</u> | 20   | 24<br>050                  | 50<br>3816           | ND.<br>ND       |  |  |
| Ty 57-70<br>Ty 58-70<br>Ty 59-71<br>Ty 60-72<br>Ty 61-75 |          | • | •           |            | · -         | -<br>-<br>- | -            | -           | -      | -      | -          | · •    | - | •      |            | •    | ND<br>ND<br>ND<br>ND<br>ND | ND<br><8<br><8<br><8 | <8<br><8<br><8  |  |  |
| 1088mel Lysate   | <b>:</b> |   |             |            |             |             |              |             |        |        |            |        |   |        |            | 6    | 762                        | >5500                | ) ND            |  |  |

TIL & EBV backgrounds for Exp.1=53, Exp.2=296, Exp.3=99 pg/ml. These have been subtracted from the numbers shown above.

Ty 56-70

QNILLSNAPLGPQFP

Tv 448-462

DYSYLQDSDPDSFQD

FIGURE 7

# CD4+ 1088 TIL RECOGNIZE MODIFIED TYROSINASE 56-70 PEPTIDES

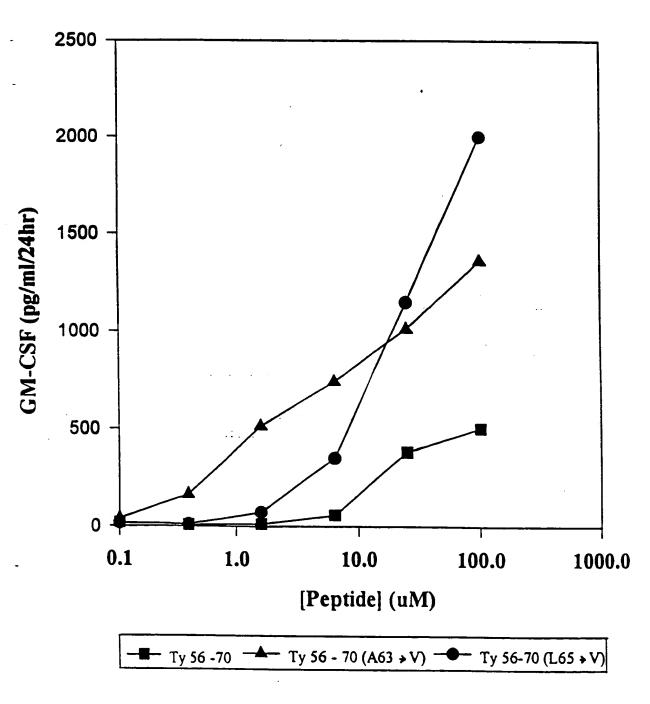


FIGURE 8

#### Determining the P1 Anchor for Ty 448-462

|  |   |        |   |        |   |   |   |   |   |   |   |   |   |        |   | GN<br>Exp. 1           | M-CSF (pg/r<br>Exp. 2    | nl/24h.) |
|--|---|--------|---|--------|---|---|---|---|---|---|---|---|---|--------|---|------------------------|--------------------------|----------|
| Ty 448-462   | D | Y      | S | Y      | L | Q | D | S | D | P | D | S | F | Q      | D | 108                    | 802                      |          |
| Y449   | - | Q<br>F | - | -      | - | - | - | - | - | - | - | • | - | -      | - | 74<br>103              | 520<br>781               |          |
| Y451   | - | -      | - | Q<br>F | - |   | - | - | - | - | • | - | - | ·<br>- | • | 28<br>324              | <8<br>1065               |          |
| L452   |   |        |   | -      |   |   |   |   | - |   |   |   |   |        |   | 14<br>14               | <8<br><8                 |          |
| Ty 449-462<br>Ty 450-462<br>Ty 451-462<br>Ty 452-462 |   | -      | - | -      | - |   | - | - | - | - | - | - | - | •      | - | 485<br>556<br>21<br>18 | 1960<br>2749<br><8<br><8 |          |
| 1088-mel Lysate                                      |   |        |   |        |   |   |   |   |   |   |   |   |   |        |   | >5500                  | 5822                     |          |

ŢIL & EBV backgrounds for Exp.1=52 and Exp.2=196 pg/ml. These have been subtracted from the numbers shown above.

### CD4+ TIL 1088 RECOGNIZE MODIFIED TYROSINASE 448-462 PEPTIDES

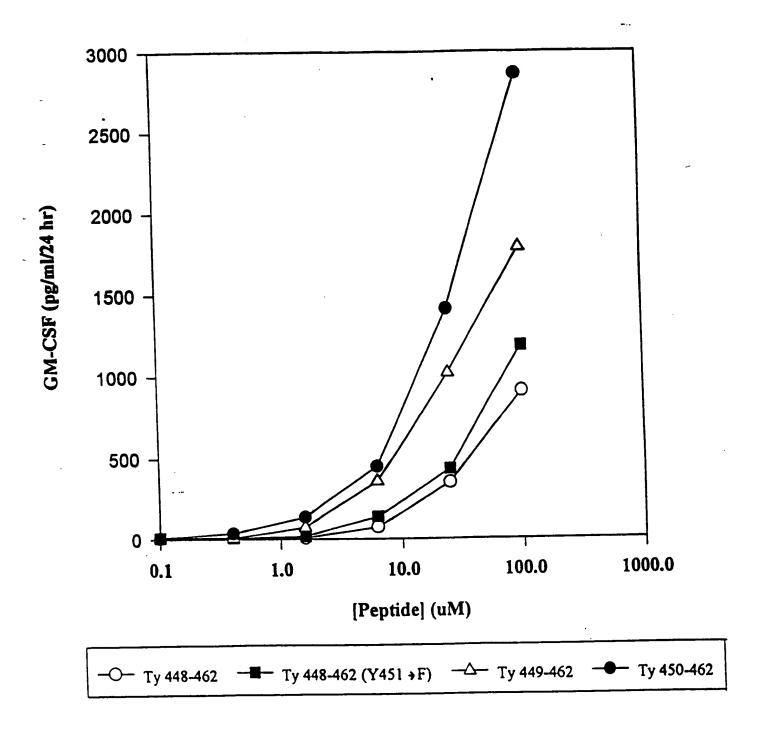


FIGURE 10

## CD4+ TIL 1088 Recognize Modified Ty 448-462

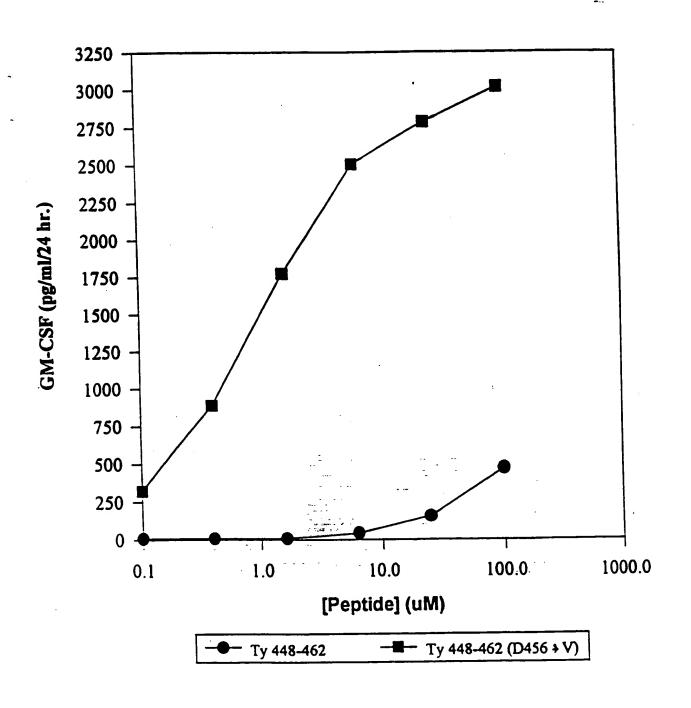


FIGURE 11

#### CD4+ TIL 1088 RECOGNIZE MODIFIED TYROSINASE 448 - 462 PEPTIDES

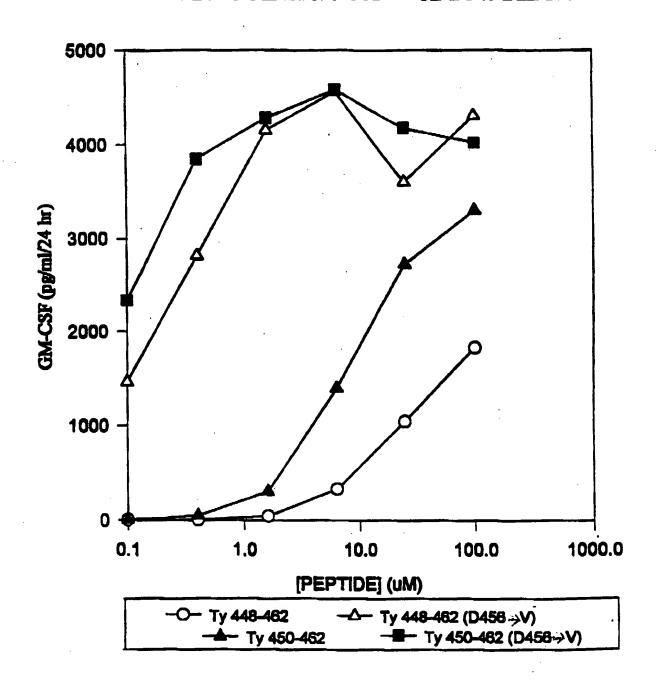


FIGURE 12